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Hyperbaric Oxygen (HBO) - Induced Toxicity; Protection by Anti-Melanogenic Compounds; Rodent Malaria Model to Test for HBO Toxicity.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Hyperbaric oxygen (HBO) is employed to treat various clinical disorders, however, its use has been limited in view of the associated toxicity to the central nervous system, lungs and circulating erythrocytes. In the present study, we assessed the efficacy of select anti-melanogenic agents to modulate the toxicity of 100% HBO in malaria infected mice since malarial parasites generate oxidants which diminish the ability of host erythrocytes to prevent and repair oxidant damage. Accordingly, it was anticipated that HBO would

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cause selective lysis of parasitized erythrocytes and hence result in a depression in parasitemia. Furthermore, any benefit derived from an effective agent would be noted by the drug's ability to diminish the severity of parasitemia decline following HBO exposure.

Female CD-1 mice (26-30 gm.) were given an intraperitoneal inoculum of 5.0 x 10^4 P. berghei-infected erythrocytes. Ten days later, mice were assayed for circulating erythrocyte and parasitemia levels and were divided into groups representing: (a) controls, (b) HBO-exposed (10 min. compression, 90 min. exposure at 3 atmospheres absolute; 45 min. decompression), (c) drug-treated and (d) drug-treated 1.5 hrs. prior to HBO exposure. Circulating erythrocytes and parasitemia levels were remonitored 24 hours after treatment. All data is based as a % of day 11 control parasitemia + 1 S.E.M.

The data clearly indicate that HBO is an effective maneuver to selectively lyse parasitized erythrocytes. In this regard, HBO effected a 20-40% depression in circulating parasitemia relative to non-exposed controls, when monitored 24 hours (on day 11) after exposure. The drug 2-thiouracil in doses of 20 to 100 mg/kg body weight (but not 10 mg/kg) were effective in combating the HBO-induced decline in parasitemia. Iso-ascorbic acid (500 mg/kg) and reduced glutathione (50 mg/kg) were likewise effective against HBO exposure. Neither drug (i.e., 2-thiouracil, iso-ascorbic acid; reduced glutathione) had any appreciable effect on the course of parasitemia in non-exposed material mice.

Our plans for future research include the following: (a) To complete the dose-response curve for 2-thiouracil at a level of 15 mg/kg body weight, (b) to perform dose-response curves for the druge, iso-ascorbic acid and reduced glutathione, (c) to assess the efficacy of the remaining 6 anti-melanogenic compounds mentioned herein, as well as others, in our sensitive model malarial system and (d) to test our potentially beneficial agents against other criteria, such as convulsion and survival times in normal non-infected mice exposed to HBO.

OFFICE OF NAVAL RESEARCH

Contract N00014-77-C-0568

Task No. NR 207-095

Terminal Report For the Period: 9/1/77 through 8/31/79

Modulation of Oxygen Toxicity by Select Anti-Melanogenic Compounds

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1. Objectives

As stated in Section F, item 2, of the Award/Contract agreement, "the Contractor shall conduct research to seek agents capable of inhibiting oxidation of catechol amines to adrenolutin, adrenochrome and rheomelanine. Specifically, mice will be inoculated with Plasmodium berghei (NK/65 strain) in a standardized fashion. The effect of hyperbaric O_2 on the progress of the infection will be studied and standardized conditions worked out. Once the limits of behavior of the infection as affected by hyperbaric O_2 are delineated, dosage response curves of the drugs will be obtained. From these curves the relative potency of the agents can be delineated. From these latter data (plus pathology studies for side effects) the most suitable drug for clinical trial of its value in the anemia of trauma or oxygen can be determined".

11. Background Information

High O_2 pressures have been employed in the treatment of several clinical disorders (1-6), but its prevalent use has been limited by the associated toxicity to the central nervous system (manifested by convulsions), lungs (characterized by hemorrhage, emphysema and atelectasis) and circulating erythrocytes (evidenced by hemolysis). The underlying mechanisms responsible for the O_2 -induced pathologies are unclear, but may relate to processes involving the metabolic generation of melanins or their immediate precursors. The biochemistry of melanogenesis is not fully understood, however, it has been shown that epinephrine, norepinephrine, dopamine and dopa are capable of forming soluble melanins in human plasma (7-9). The role of melanogenesis in the pathology of O_2 toxicity is strengthened by the finding of increased adrenochrome production and tissue deposition during hyperbaric O_2 exposure (10). Furthermore, adrenochrome and adrenolutin have been implicated in a number of neurological disorders (11) and have been shown to induce hemolysis (12). Plasma-soluble melanins are likewise toxic to circulating red cells (12; 13), and probably to bone marrow

cells as well (14). Accordingly, it is most probable that the anemia associated with high oxygen tension is due, at least in part, to the effects of soluble melanins or their immediate precursors.

Another syndrome that appears to be related to plasma-soluble melanins or their immediate precursors, is the unexplained anemia of trauma (15; 16). The disorder is characterized by chronic deficits in total red cell-hemoglobin mass, as a consequence of depressed erythropoiesis and accelerated erythrocyte destruction (15-18). Although the specific etiology of stress anemia is unclear, it is speculated that the presence of one or more toxic factors is responsible for the inhibition in erythropoiesis and the enhanced destruction of circulating red cells. In this regard, several reports have shown that red cells from such patients have an increased in vitro susceptibility to the toxic effects of adrenochrome (16, 18; 19).

It is of interest to note that in our studies concerning virulent rodent malaria (P. berghei), we have (20-22) shown strikingly similar erythroid patterns and responses as those reported in stress anemia. Of further significance are the reports which show that the malaria parasite generates oxidants within infected erythrocytes, which, in turn, diminish the ability of the host cells to prevent or repair oxidant damage (23). In this regard, exogenous oxidant drugs (24) and hyperoxia (25) will cause the premature lysis of malarial erythrocytes, thereby limiting the severity of infection.

In summary: (a) one of the consequences of O_2 toxicity is increased hemolysis and the development of anemia; an occurrence which seems to be mediated by increased availability of melanin or one or more of its immediate precursors; (b) the anemia associated with O_2 toxicity bears a strong resemblance to that seen in victims of trauma as well as malaria (P. berghei) -infected mice; (c) P. berghei parasites exert an oxidant stress on the host

erythrocyte (i.e. erhanced oxidation of NADH, NADPH, GSH and hemoglobin (Fe++) and generation of H_2O_2), which consequently decrease the ability of infected red cells to repair oxidant damage; thereby rendering them highly susceptible to hemolysis. Accordingly, we propose that the P. berghei-infected mouse erythrocytes afford a highly sensitive model system by which to monitor the toxic effects (i.e. selective hemolysis of parasitized erythrocytes) of hyperbaric O_2. Furthermore, this system can be employed to test the efficacy of antimelanogenic agents to combat the pathologies associated with O_2 poisoning, by monitoring the drug's ability to prevent or diminish the severity of parasitemia decline following O_2 exposure. In this regard, we have enjoyed collaborating with Dr. Mark D. Altschule of Harvard Medical School, who has provided us with a list of select anti-melanogenic agents (i.e. disulfiram, 2-thiouracil, D-penicillamine, ascorbic acid, isoascorbic acid, butylated hydroxytoluene, 3,3'-propyl dilauryl thiodipropionate, thiosalicylic acid and 2-mercaptobenzothiazole), which have been shown to inhibit (in vitro) the hemolysis associated with stress anemia.

III. Research Progress

A. General Comments

Many problems and variables were encountered in establishing an effective protocol for studying hyperbaric O_2 toxicity in malaria mice. In brief, our concern was directed toward: (a) construction of a safe, gas-tight hyperbaric chamber, (b) establishment of appropriate O_2 flushing, compression, exposure and decompression procedures, (c) selection of an optimized malarial mouse test system as related to: body weight, level of infection (Plasmodium berghei) inoculum, day of infection on which to expose; level and duration of hyperbaric O_2 exposure, and (d) determination of appropriate time intervals at which to assess the consequences of O_2 toxicity.

Through persistent experimentation, we have established what we consider to be a standardized and effective protocol. Furthermore, based on this scheme, we have undertaken studies to assess the efficacy of several anti-melanogenic compounds to block O_2 toxicity. The details of our studies are provided in the subsequent sections of this report.

B. Hyperbaric Oxygen Chamber

After several unsuccessful attempts to construct a safe, gas-tight hyperbaric chamber (from various materials including plexiglass and lucite), we chose to modify a conventional 20-liter capacity aluminum alloy pressure sterilizer for this purpose. The sterilizer had previously been purchased by my Department from Wisconsin Aluminum Foundry Co., Inc., Manitowoc, Wisconsin, hence only minor costs were required to make the appropriate adjustments and modifications for conversion. In this regard, to the three openings in the original sterilizer lid, we attached a pressure gauge (0 to 60 L/min.) and an O_2 intake connector pipe. With reference to the latter, it should be emphasized that all incoming O_2 is bubbled through a water bath in the bottom of the chamber (caged mice rest on a plexiglass platform), so that proper hydration of the gas is accomplished prior to inspiration by the mice. A KOH solution, containing fluted filter paper, is also present for the absorption of expired CO_2. The chamber has proven to be suitable for housing up to 12 mice comfortably. Lastly, in accord with specifications established by the manufacturer and consultation with Lowell University's Mechanical Engineering Department, the chamber has been deemed completely safe and gas-tight at the desired levels of hyperbaric exposure. These aspects have been confirmed in several preliminary and many subsequent experiments, in which we maintained constant chamber pressure (2-4 atmospheres, absolute) for up to six hours without incident.

C. Conditions of Oxygen Exposure

1. Chamber Flushing

Immediately after mice are placed in the hyperbaric chamber and the lid tightly secured (with exhaust regulator valve maximally open), 100% O_2 is flushed through the chamber for 5 minutes at the rate of 30 L/minute. In accord with the formula: $R=\frac{FR}{V}$, where: R equals the fraction of gas mixing/unit time, FR equals the flow rate (30 L/min.) of gas (O_2) through the chamber and V equals the chamber volume (20L), the T/2 (time for 50% mixing of 100% O_2 and chamber air) calculates to be 27.6 seconds. Accordingly, based on an exponential function, after 5 minutes of flushing, approximately 99.96% of the gaseous chamber environment will consist of pure O_2, not including the volume occupied by water vapor during hydration.

2. Compression

Subsequent to the initial 5 minute O_2 flushing procedure, the exhaust regulator valve on the chamber lid is progressively tightened at a rate of 2 p.s.i./minute until a specified hyperbaric pressure is gradually reached over a 10-minute interval. Immediately thereafter, the gas flow regulator on the O_2 tank is closed in order to prevent further O_2 inflow and hence unwanted pressure elevation within the chamber. Although the gradual (10 minute) compression scheme that we employ is somewhat longer than that (3-8 minutes) reported elsewhere (26, 27), it has proven to be a most effective maneuver in minimizing animal fatality in our studies.

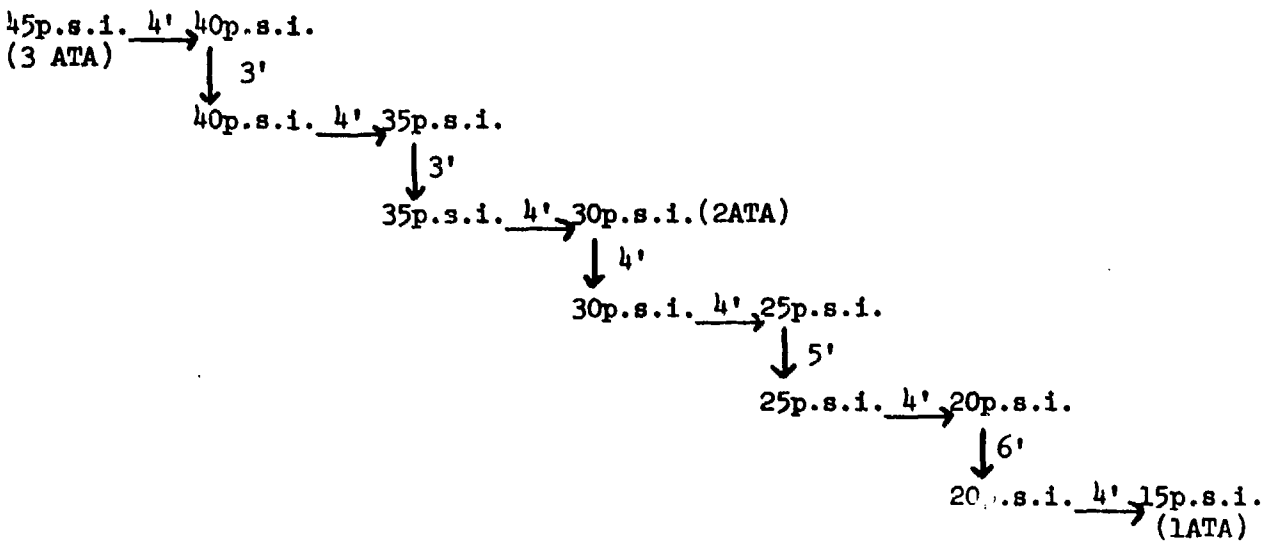
We have performed many preliminary experiments in which we subjected mice to hyperbaric oxygen, ranging from 2 to 4 atmospheres, absolute (i.e. 30-60 p.s.i.). Based on these experiments, we have determined that 3 atmospheres, absolute (45 p.s.i.) is the optimum level of oxygen pressure to employ, from the standpoint of yielding desired end-effect (i.e. parasitemia depression) and respectable survival of exposed mice.

3. Duration of Exposure

Experimentation has revealed that 1.25 hours of exposure (at 3 atmospheres, absolute) is an adequate interval of time for the development of oxygen-induced toxicity in malarial mice (as monitored by selective hemolysis of parasitized erythrocytes). In this regard, shorter intervals have resulted in a lesser degree of hemolysis, while extending the duration of exposure (up to 6 hours) has diminished survival of malarial mice to as low as 0%. Accordingly, the 1.25 - hour exposure is most effective when considering the aspects of high perturbation and respectable mortality.

4. Decompression

At the termination of the 1.25 - hour hyperbaric oxygen exposure, the exhaust regulator valve on the chamber lid is progressively opened to allow for a step-wise decompression over a 45-minute interval. In our initial studies, shorter decompression schemes, as reported elsewhere (28), have drastically affected survival of our malarial mice. The specific decompression scheme is presented below:



Based on the durations of O_2 exposure during chamber flushing (5'), compression (10'), HBO exposure (1.25 hr.) and decompression (45'), all mice are subject to approximately 2 hours of O_2 throughout.

D. Status of Malarial Mice

In the past we reported the course of parasitemia and anemia in malarial mice which had received an inoculum of P. berghei-infected erythrocytes (20, 21). Based on these and other studies (as reviewed in Ref. 29), it is firmly established that the course of malaria infection is influnced by many variables. In this regard, we sought to develop a reproducible animal system which would be suitable in the study of hyperbaric O_2 toxicity. Accordingly, through preliminary experiments, we have learned that optimization is achieved when CD-1 female mice, 23-27 g. body weight are infected i.p. with an inoculum containing 5.0×10^4 parasitized erythrocytes and exposed to oxygen 10 days thereafter (when parasitemias are usually in the range of 5 - 10%). Oxygen exposure at day 10 is favorable from the standpoint of circumventing the low parasitemias and high mortality associated with earlier and more progressed infections, respectively.

E. Assessment of Exposed Mice

In preliminar experiments, we monitored the numbers of circulating erythrocytes and % parasitemia (from which we calculate the numbers of parasitized erythrocytes) in malarial mice, prior to and at many intervals (up to 96 hours) following O_2 exposure. As a result, we determined that such measurements, immediately prior to and at 24, 48 and 72 hours after O_2 exposure were adequate in defining the limits of behavior of malarial infection as affected by hyperbaric O_2. Data derived from non-exposed infected controls (Table 1) are reported as a % of day 10 values and are used as the basis of comparison for experimental groups treated with drugs and/or O_2. This aspect is critical when one realizes that infected non-exposed mice develop progressive increases in levels of circulating parasites throughout the 4-day assay period. In brief, comparison

of parameters between experimental and non-exposed infected mice gives a more accurate account of the toxicity (i.e. hemolysis) associated with hyperbaric O_2 exposure.

F. Outline of Standardized Protocol (Diagramatically outlined in Figure 1)

Day 0 - Adult, female, CD-1 mice, 23-27 g body weight, are infected with an i.p. inoculum containing 5.0×10^4 parasitized (P. berghei) erythrocytes.

Day 10 - Infected mice are divided into the following groups: (a) non-exposed, (b) non-exposed drug treated, (c) exposed and (d) exposed drug-treated.

- All mice are assessed for peripheral parasitemia and erythrocytes (tail blood).

- Drug-treated groups (b and d) are injected 1-2 hours prior to O_2 exposure.

- Appropriate groups (c and d) are exposed to 100% hyperbaric O_2 (3 atmospheres, absolute) for approximately 2 hours (as described in Section III C) and survival recorded thereafter.

Days 11, 12, 13 All groups of mice are assessed for peripheral parasitemia and erythrocytes and survival recorded.

- All experimental data (erythrocytes and parasitemia) are expressed as a % of daily levels in infected non-exposed (group a) mice. In our updated scheme, determination of % parasitemia alone is of significance since red cell enumeration may lead to erroneous findings.

G. Experimental Findings

1. Normal Course of Malarial Infection

Table 1 shows the normal course of anemia and parasitemia in malarial mice, based as a % of day 10 values. In general, it can be noted that malarial mice intensify their anemic and parasitemic status as they progress from day 10 to day 13 of infection. During this period, approximately 11% of the 18 mice succumbed to the deleterious consequences of infection, resulting in 16 survivors by day 13.

2. Course of Infection Following Oxygen Exposure

Table 2 demonstrates the effect of hyperbaric oxygen on the erythroid and parasitemic status of malarial mice. As noted, the levels of circulating erythrocytes are slightly elevated (2-12%), relative to infected non-exposed controls. Based solely on this finding, it would appear that hyperbaric oxygen exposure is ineffective in inducing hemolysis. However, in view of the approximately 40-45% decrease in % parasitemia and 32-44% depression in absolute parasitemia, it is clear that this treatment does indeed result in the selective elimination of parasitized erythrocytes as discussed in Section II of this report. The rationale for the role of elevations in in circulating erythrocytes is unclear, but may perhaps reflect plasma loss, resulting in hemoconcentration and the consistently higher absolute parasitemias throughout. The effect of HBO on the course of parasitemia of malarial mice is diagramatically shown in Figure 2. Lastly, since the decline in parasitemia on day 11 is representative of all days assayed, a single determination (on day 11) was deemed appropriate for drug assessment in our latter experiments.

3. Effect of Drugs

a. Disulfiram

Table 3 shows the effect of disulfiram (20 mg/kg, i.p.) on the course of anemia and parasitemia in infected control and oxygen-exposed (day 10) mice. The data clearly show that disulfiram was totally effective in preventing the oxygen-induced decline in % parasitemia (days 11 and 12). The seemingly lack of protection noted on day 13 is most likely attributed to the effects of disulfiram treatment per se on parasitized erythrocytes - especially when one notes the depressions in parasitemia produced by this agent in the non-exposed group. Perhaps the use of disulfiram in lower doses would minimize these changes and still be effective against the toxicity associated with hyperbaric oxygen. In this regard, we plan to repeat this study with larger groups of mice and additionally perform dosage-response curves.

b. D-Penicillamine

Table 4 shows the effect of D-Penicillamine (10 mg/kg, i.p.) on the course of anemia and parasitemia in infected control and oxygen-exposed (day 10) mice. The data show that this agent enhances the anemic status of infected mice. In non-exposed mice, the drug also is responsible for depressing the level of parasitemia to approximately 50% of control levels on day 13. In view of these confounding changes, any possible beneficial effect that D-penicillamine may have had in combating oxygen toxicity were undoubtedly masked. Nevertheless, minimal protection is noted when one compares the decline in parasitemia to 65.8% on day 11 in drug-treated mice, as opposed to 57.5% in the infected pooled control groups (Table 2). Perhaps an alteration in dosage and/or assessment of mice at shorter intervals after O_2 exposure will prove advantageous. Obviously, additional studies are required before a judgment can be made relative to the value of D-penicillamine.

c. 2-Thiouracil

Figure 3 shows the effects of various doses of 2-Thiouracil (10, 20, 30, 40 and 50 mg/kg, i.p.) on the parasitemia of malaria-infected HBO exposed mice. The following conclusions are supported by these data: In a dose of 20, 30, 40, or 50 mg/kg, 2-thiouracil is significantly effective ($p<0.05$) in blocking HBO - induced toxicity (i.e. this drug prevents the decline in parasitemia) in our model malaria system, while 10 mg/kg is virtually ineffective. Additional studies are being performed using 15 mg) kg of 2-thiouracil to further delineate the pattern of dose responsiveness. Also, please refer to abstract 1 enclosed herein.

d. Isoascorbic Acid

Figure 4 shows the effects of isoascorbic acid (500 mg/kg, i.p.) on the parasitemic levels of malarial and malarial HBO-exposed mice. It is clear from this study that isoascorbic acid has no effect per se on the parasitemic levels of malarial mice, and is completely effective in blocking HBO - induced

toxicity, as manifested by the prevention of parasitemia decline following HBO exposure. Please refer to abstract 2 for further clarification.

e. Glutathione

Figure 5 shows the effect of reduced glutathione (60 mg/kg, i.p.) on the circulating parasitemia levels of malarial and malarial HBO-exposed mice. From these data, it is apparent that while GSH has no influence on the course of parasitemia of infected mice, it is totally effective in blocking HBO-induced toxicity in our malarial model system.

f. Ascorbic Acid

Figure 6 represents preliminary data on the efficacy of ascorbic acid to block HBO-toxicity. Although it would appear that this agent, in a dose of 500 mg/kg (ID group) may exert some depressive effect on circulating parasitemia levels, it nevertheless was quite effective in preventing the decline in parasitemia subsequent to HBO exposure.

IV. Concluding Remarks

Through experimentation, as described herein, we have been successful in developing an effective protocol by which to test the relative potency of select anti-melanogenic drugs in hyperbaric O_2 toxicity (and the anemia of trauma). In this regard, we have devised a safe and inexpensive hyperbaric O_2 chamber and have delineated the conditions for exposing and assessing malarial mice at appropriate phases of infection. Based on this scheme, we have demonstrated that certain drugs tested have varying ability to block HBO-toxicity, as assessed in our model malarial system. In this regard, it would appear that: (a) disulfiram is effective (Table 3), (b) D-penicillamine has questionable benefit (Table 4), (c) 2-thiouracil is most effective (Figure 3; abstract 1), (d) isoascorbic acid is most effective (Figure 4; abstract 2), (e) glutathione (reduced) is most effective (Figure 5), and (f) ascorbic acid has totential efficacy (Figure 6). Obviously additional studies are required to define the dose-response

of most of the drugs already tested and to expand the list of potentially effective drugs.

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VI. Appendix

Table I

Peripheral Erythroid and Parasitemic Status of Non-Exposed *P. berghei*-infected Mice

Erythrocytes

Exper./(No. Mice)	Day of Infection 11	12	13
5 (5)	(5) 78.1	(5) 63.1	(5) 59.4
8 (4)	(4) 82.0	(4) 69.3	(4) 50.3
9 (6)	(6) 83.8	(6) 65.6	(4) --
10 (3)	(3) 74.6	(3) 62.4	(3) 57.6
Mean:	80.3	65.2	56.5

% Parasitemia

Exper./(No. Mice)	Day of Infection 11	12	13
5 (5)	(5) 168.8	(5) 148.7	(5) 243.9
8 (4)	(4) 140.9	(4) 208.3	(4) 387.0
9 (6)	(6) 143.2	(6) 199.5	(4) --
10 (3)	(3) 139.6	(3) 184.6	(3) 254.0
Mean:	149.2	184.9	294.1

Absolute Parasitemia

Exper./(No. Mice)	Day of Infection 11	12	13
5 (5)	(5) 131.8	(5) 93.8	(5) 144.9
8 (4)	(4) 115.5	(4) 144.4	(4) 194.7
9 (6)	(6) 120.0	(6) 130.9	(4) --
10 (3)	(3) 104.1	(3) 115.2	(3) 146.3
Mean:	119.6	121.0	161.9

- Adult, female, CD-1 mice, 23-27 g body weight were infected on day 0 with an i.p. inoculum containing 1.0×10^5 parasitized erythrocyies.
- Values in parenthesis reflect the numbers of mice.
- Experimental values for days 11, 12 and 13 are based as a % of day 10 levels.
- Mouse survival ranged from 67 - 100%, with a mean of 89% for these experiments.

Table 2

Peripheral Erythroid and Parasitemic Status of *P. berghei*-Infected Mice Following Hyperbaric Oxygen Exposure

Erythrocytes

Exper./(No. Mice)		Day of Infection 11	12	13
5	(5)	(5) 103.3	(5) 123.8	(5) 114.3
8	(4)	(2) 81.4	(2) 77.9	(2) 93.6
10	(6)	(5) 116.9	(5) 115.5	(5) 93.2
Mean:		105.3	112.7	102.1

% Parasitemia

Exper./(No. Mice)		Day of Infection 11	12	13
5	(5)	(5) 45.3	(4) 61.6	(4) 53.3
8	(4)	(2) 71.2	(2) 59.0	(2) 39.6
10	(6)	(5) 64.3	(5) 59.9	(5) 64.4
Mean:		57.5	60.4	55.9

Absolute Parasitemia

Exper./(No. Mice)		Day of Infection 11	12	13
5	(5)	(5) 46.8	(4) 76.3	(4) 60.9
8	(4)	(2) 58.0	(2) 46.0	(2) 37.1
10	(6)	(5) 75.2	(5) 69.2	(5) 60.0
Mean:		60.5	67.6	56.2

- Adult, female, CD-1 mice, 23-27 g body weight were infected on day 0 with an i.p. inoculum containing 1.0×10^5 parasitized erythrocytes.
- Values in parentheses reflect the numbers of mice
- Mice were subjected to hyperbaric oxygen (3 atmospheres, absolute) for 2 hours, on day 10 of infection.
- Experimental values for days 11, 12 and 13 are based as a % of daily levels of non-exposed infected controls.
- Mouse survival ranged from 50 - 83%, with a mean of 73% for these experiments.

Table 3

Effect of Disulfiram on Peripheral Erythroid and Parasitemic Status of P. berghei-Infected Control (ID) and Hyperbaric Oxygen-Exposed (IDO) Mice

Erythrocytes
Day of Infection

Exper./Group/(No. Mice)			11	12	13
10	ID	(3)	(3) 101.2	(3) 105.6	(3) 96.5
10	IDO	(5)	(3) 113.7	(3) 125.2	(3) 92.9

% Parasitemia
Day of Infection

Exper./Group/(No. Mice)			11	12	13
10	ID	(3)	(3) 99.1	(3) 86.9	(3) 74.1
10	IDO	(5)	(3) 109.7	(3) 102.4	(3) 63.7

Absolute Parasitemia
Day of Infection

Exper./Group/(No. Mice)			11	12	13
10	ID	(3)	(3) 100.3	(3) 91.8	(3) 71.5
10	IDO	(5)	(3) 124.7	(3) 128.2	(3) 59.2

- Adult, female, CD-1 mice, 23-27 g body weight were infected on day 0 with an i.p. inoculum containing 1.0×10^5 parasitized erythrocytes.
- Values in parenthesis reflect the numbers of mice.
- On day 10 of infection, drug-treated mice were given a single i.p. injection of disulfiram (20 mg/kg) 4 hours prior to subjecting one group to hyperbaric oxygen (3 atmospheres, absolute) for 2 hours.
- Experimental values for days 11, 12 and 13 are based as a % of daily levels of non-exposed, non-drug-treated infected controls (Table 1).

Table 4

Effect of D-Penicillamine on Peripheral Erythroid and Parasitemic Status of P. berghei-Infected (IP) and Hyperbaric Oxygen-Exposed (IPO) Mice

Erythrocytes

Day of Infection

Exper./Group/(No. Mice)			11	12	13
8	IDP	4	(4) 91.3	(4) 92.9	(4) 84.7
8	IDPO	4	(3) 78.6	(3) 98.0	(3) 129.6

% Parasitemia

Day of Infection

Exper./Group/(No. Mice)			11	12	13
8	IDP	4	(4) 104.5	(4) 87.2	(4) 52.7
8	IDPO	4	(3) 65.8	(3) 41.8	(3) 29.3

Absolute Parasitemia

Day of Infection

Exper./Group/(No. Mice)			11	12	13
8	IDP	4	(4) 95.4	(4) 81.0	(4) 44.6
8	IDPO	4	(3) 51.7	(3) 41.0	(3) 38.0

- Adult, female, CD-1 mice, 23-27 g body weight were infected on day 0 with an i.p. inoculum containing 1.0×10^5 parasitized erythrocytes.
- Values in parenthesis reflect the numbers of mice.
- On day 10 of infection, drug-treated mice were given a single i.p. injection of D-penicillamine (10 mg/kg) 1 hour prior to subjecting one group to hyperbaric oxygen (3 atmospheres, absolute) for two hours.
- Experimental values for days 11, 12 and 13 are based as a % of daily levels of non-exposed, non-drug-treated infected controls (Table 1).

DIAGRAMATIC PROTOCOL

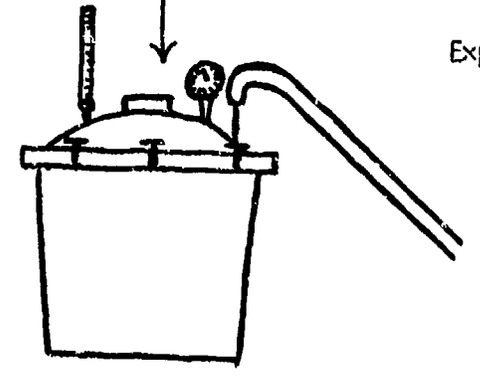
D-O

Infected with 5.0×10^4 PRBC P. berghei

D-10

Peripheral parasitemia and erythrocytes were assessed

Drug injected 1 and 1/2 hr. prior to HBO (3ATA ~2 hours)



Experimental groups

a) non-exposed

b) non-exposed drug treated

c) exposed

d) exposed drug treated

D-11

All groups were reassessed for peripheral parisitemia and erythrocytes.

Figure 1

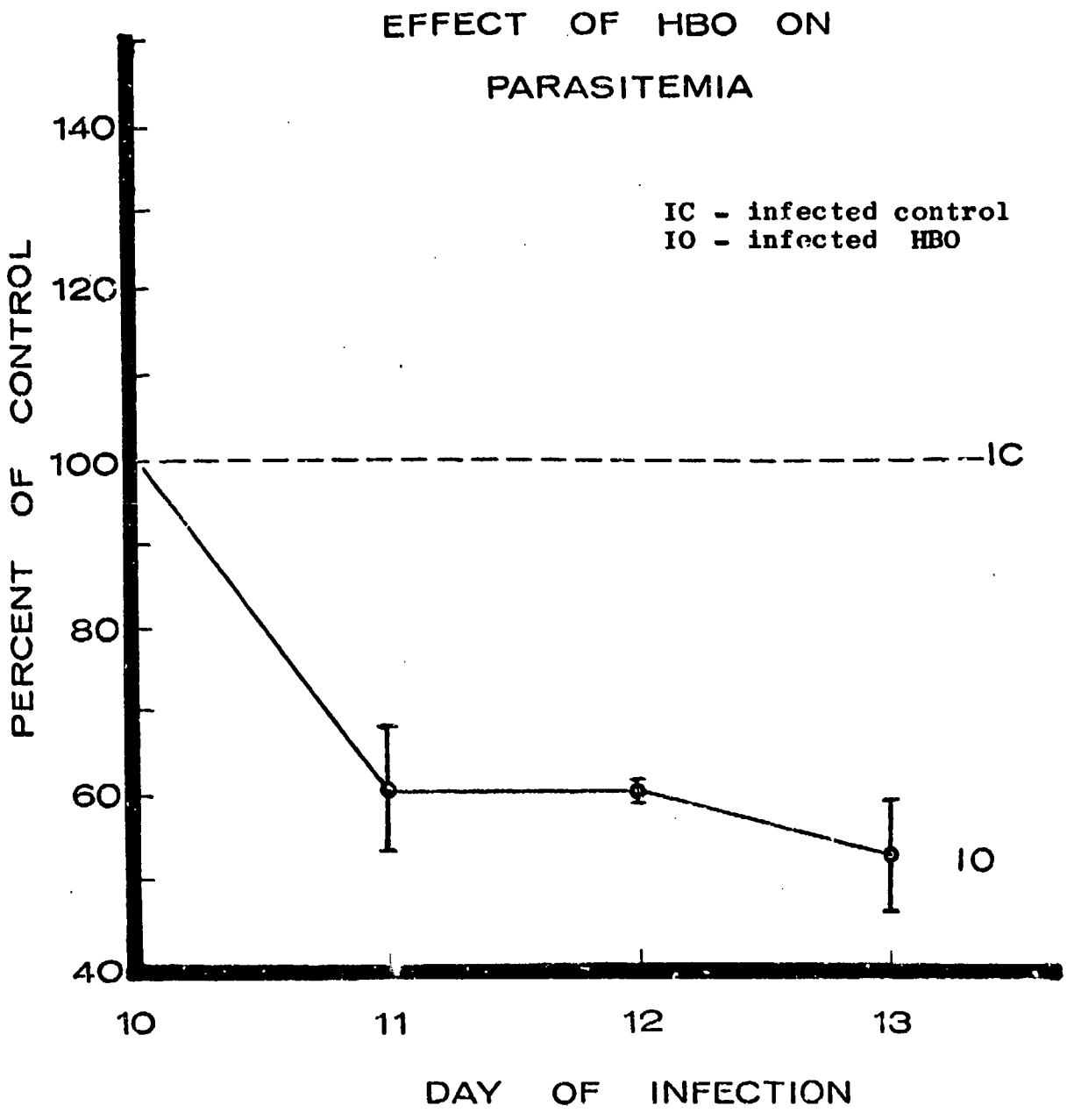
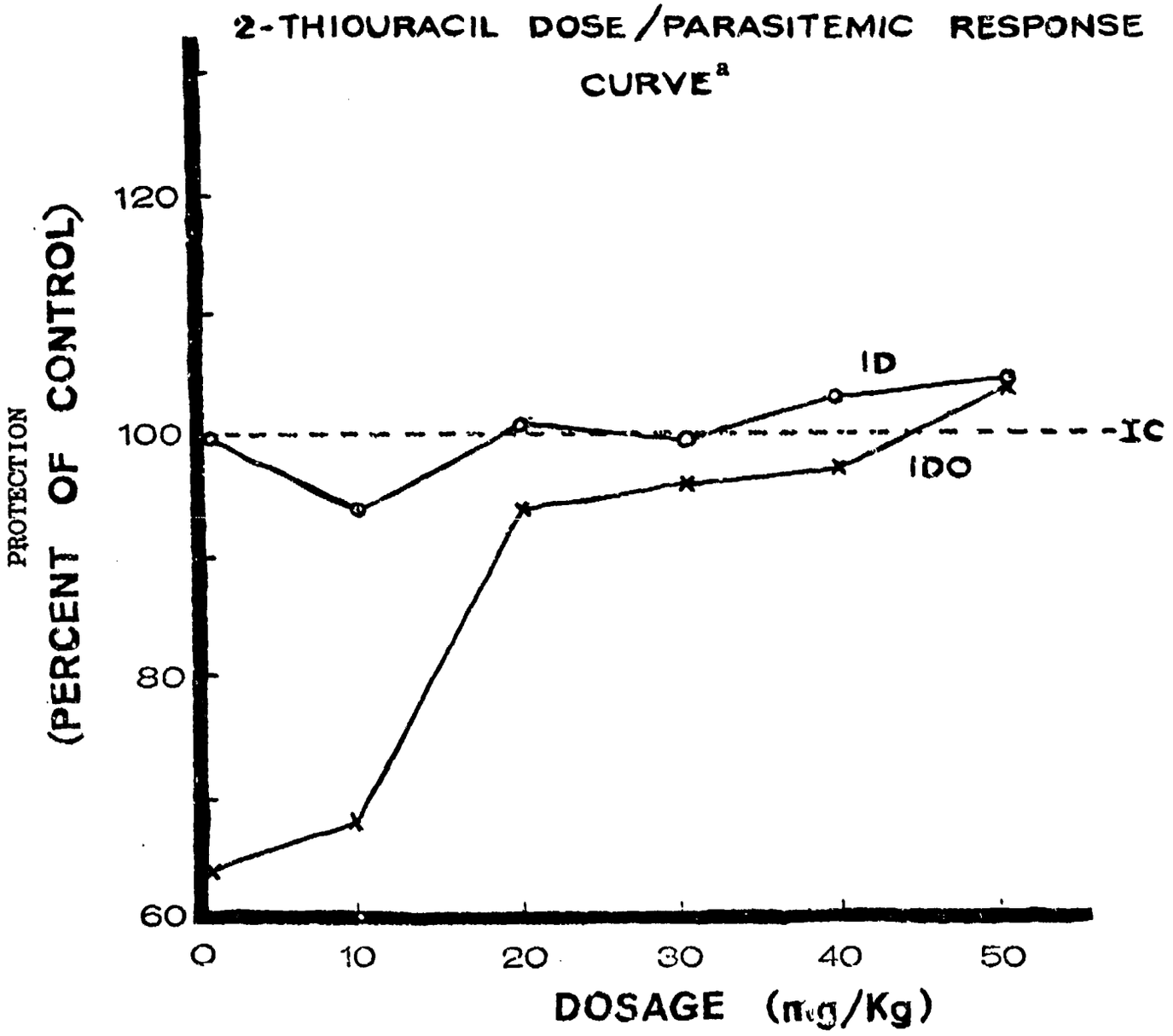

EFFECT OF HBO ON
PARASITEMIA
IC - infected control
IO - infected HBO
PERCENT OF CONTROL
140
120
100
80
60
40
IC
IO
10
11
12
13
DAY OF INFECTION

Figure 2



[a]2-thiouracil was administered ip 1.5 hours prior to HBO exposure on day 10.

Figure 3

EFFECT OF ISOASCORBIC ACID
ON PARASITEMIA OF HBO EXPOSED MICE

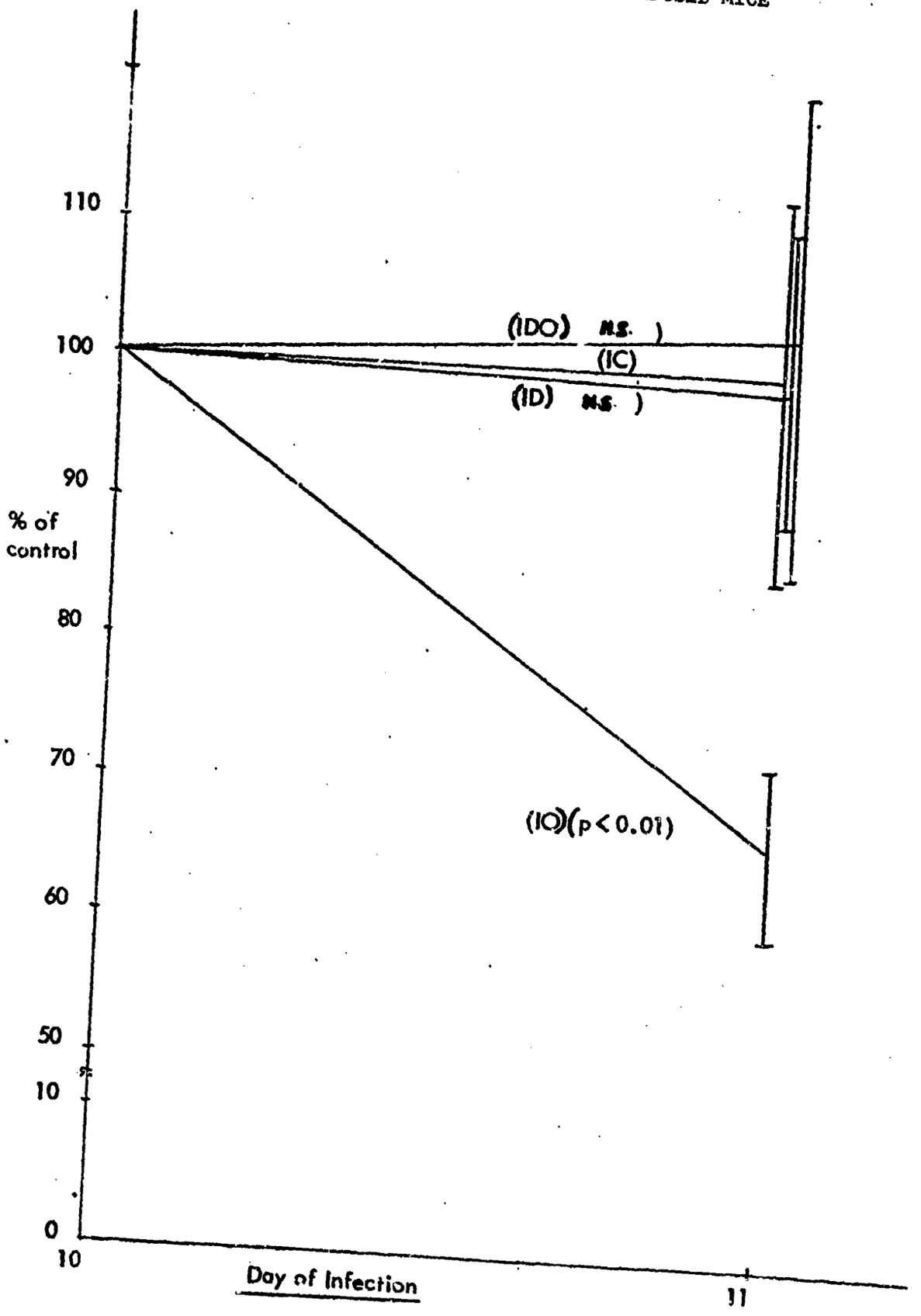
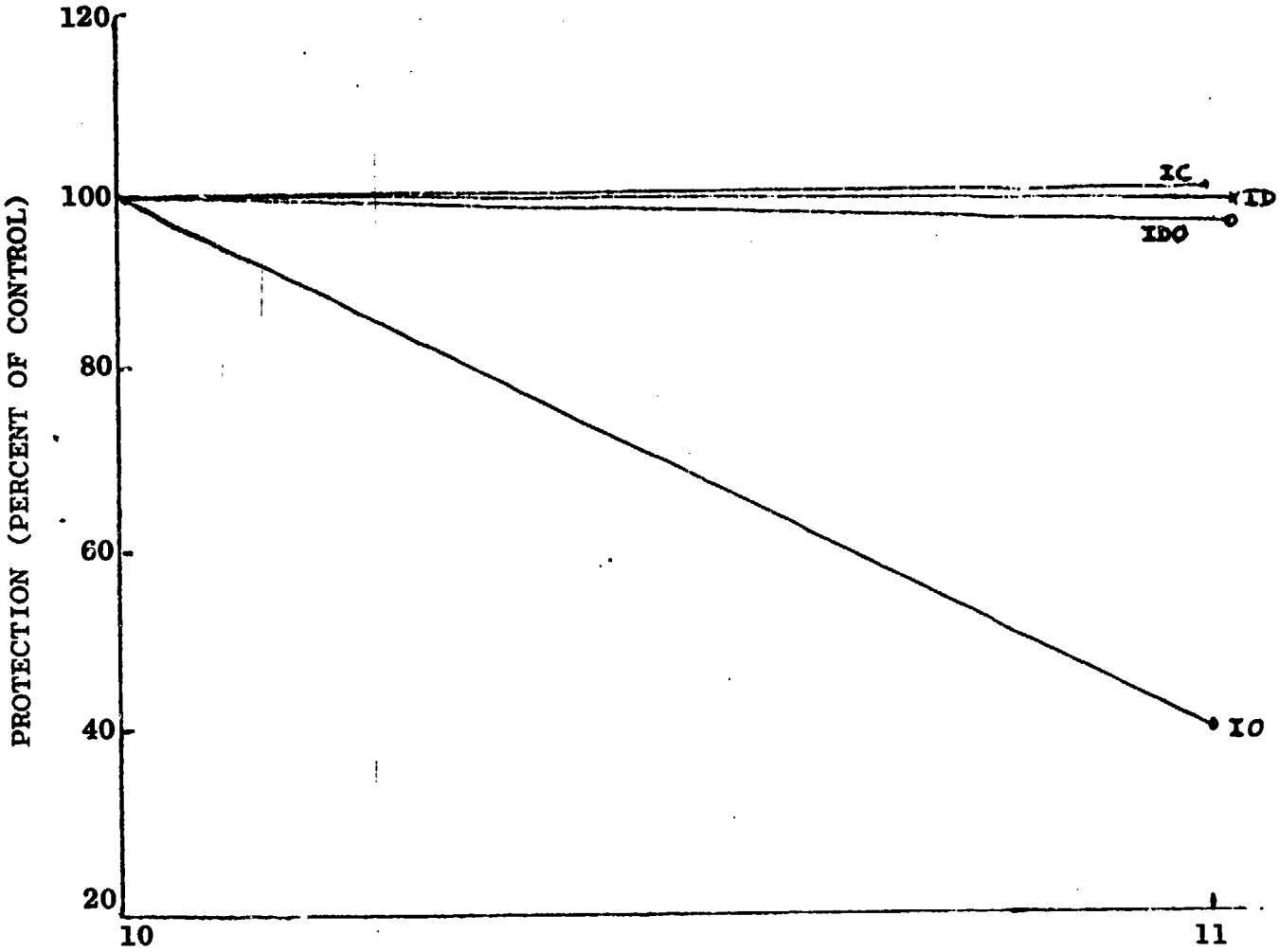


Figure 4

EFFECT OF 60 mg/kg GLUTATHIONE(GSH)[a] ON PARASITEMIA

IC - infected control
ID - infected drug
IO - infected HBO
IDO - infected drug HBO

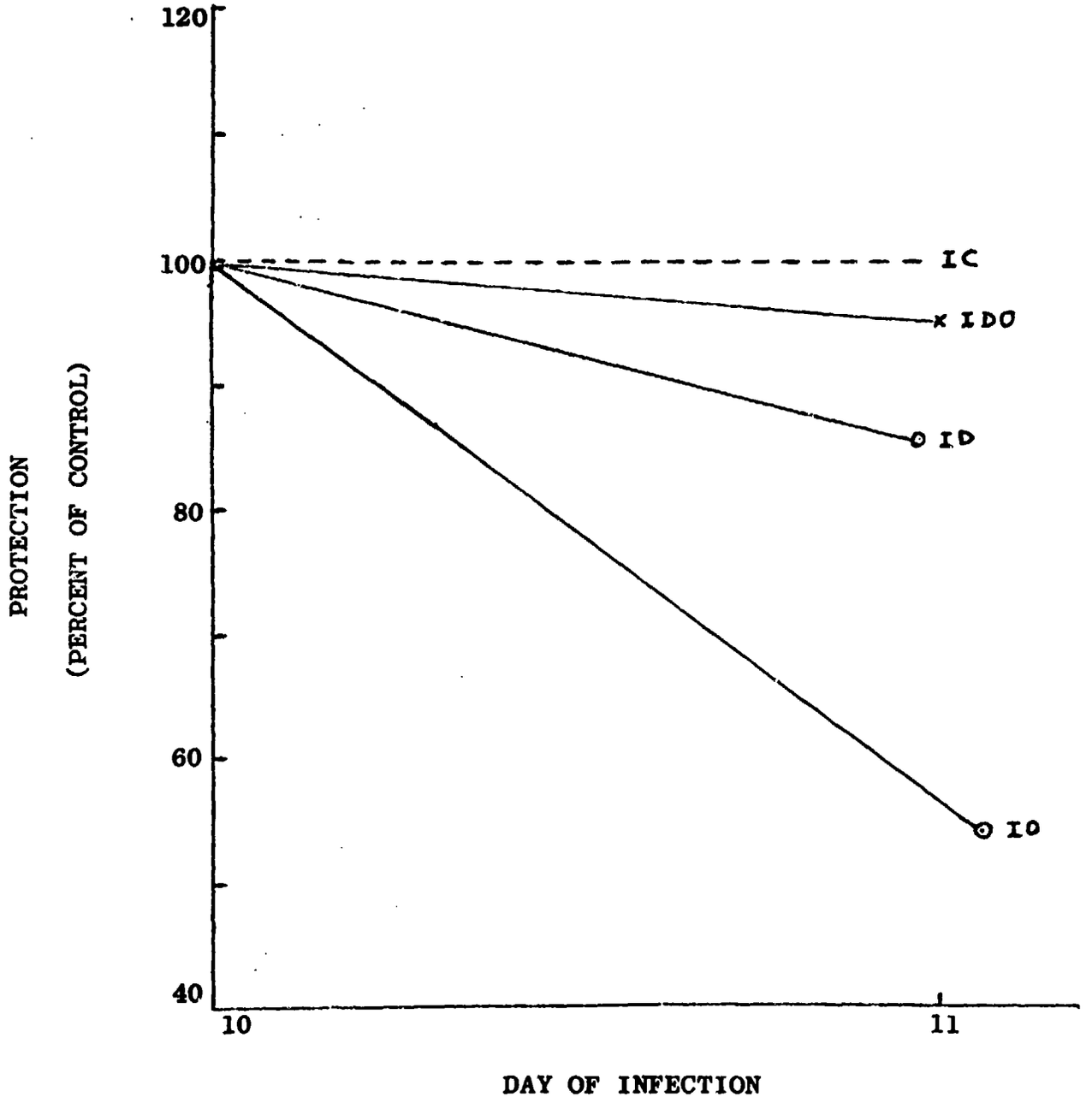


[a]GSH was administered ip 1.5 hours prior to HBO exposure on day 10.

Figure 5

EFFECT OF 500 mg/kg ASCORBIC ACID[a] ON PARASITEMIA

IC - infected control
ID - infected drug
IO - infected HBO
IDO - infected drug HBO



[a]Ascorbic acid was administered ip 1.5 hours prior to HBO exposure on day 10.

Figure 6

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Hyperbaric oxygen (HBO) is employed to treat various clinical disorders, however its use is limited in view of the associated toxicity. In the present study, we assessed the efficacy of 2-thiouracil to modulate the toxicity of 100% HBO in malaria-infected mice since malarial parasites generate oxidants which diminish the ability of host erythrocytes to prevent and repair oxidant damage. Accordingly, it was anticipated that HBO would cause selective lysis of parasitized erythrocytes and hence a depression in parasitemia. Furthermore, any benefit derived from an effective agent would be noted by the drug's ability to diminish the severity of parasitemia decline following HBO. Female CD-1 mice were given an inoculum of 5×10^4 P. berghei-infected erythrocytes. Ten days later, mice were assayed for circulating red cell and parasitemia levels and were divided into groups representing: controls, HBO-exposed (3 atmospheres, absolute for 2 hours), 2-thiouracil-treated (50 and 100 mg/kg) and 2-thiouracil-treated prior to HBO exposure. Circulating red cell and parasitemia levels were remonitored 24 hours after treatment. Mice exposed to HBO exhibited 20-35% depressions in parasitemia, relative to non-exposed controls ($p < .05$). Those given 2-thiouracil were afforded virtually total protection from HBO ($p < .05$) as revealed by minimal (2-5%) declines in parasitemia. We conclude that our malarial model is a highly sensitive system by which to assess the ability of select agents to combat HBO-induced toxicity. In this regard, we have shown that 2-thiouracil has excellent protective value against the toxicity of HBO exposures.

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